M.H

PCT/GB99/02205

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 02 March 2000 (02.03.00)	
International application No. PCT/GB99/02205	**Applicant's or agent's file reference** NW/6943INT
International filing date (day/month/year) 09 July 1999 (09.07.99)	**Priority date** (day/month/year) 10 July 1998 (10.07.98)
Applicant ALA'ALDEEN, Diawer et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

05 February 2000 (05.02.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Olivia RANAIVOJAONA
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Form PCT/IB/331 (July 1992)

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PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
NW/6943INT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB99/02205	09/07/1999	10/07/1998
International Patent Classification (IPC) or national classification and IPC C12N5/08		
Applicant THE UNIVERSITY OF NOTTINGHAM et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 16 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which hav been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 9 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☒ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
05/02/2000	06.10.00
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Valcarcel, R Telephone No. +49 89 2399 2368

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/02205

I. Basis of th r port

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-19 as originally filed

Claims, No.:

1-79 with telefax of 21/09/2000

Drawings, sheets:

1/1 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☒ the claims, Nos.: 80
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

see separate sheet

II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

   ☐ copy of the earlier application whose priority has been claimed.

   ☐ translation of the earlier application whose priority has been claimed.

2. ☒ This report has been established as if no priority had been claimed due to the fact that the priority claim has

been found invalid.

Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

see separate sheet

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 69,72-79.

because:

☒ the said international application, or the said claims Nos. 69 (with respect to industrial applicability) relate to the following subject matter which does not require an international preliminary examination (*specify*):

see separate sheet

☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 72-79 are so unclear that no meaningful opinion could be formed (*specify*):

see separate sheet

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☒ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:

see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☐ all parts.

☒ the parts relating to claims Nos. 1-26,58,59,63,65,67,70 (all completely); 64,69 (both partially).

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-26,58,59,63,65,67,70 (all completely); 64,69 (both partially)
	No:	Claims	NONE
Inventive step (IS)	Yes:	Claims	1-26,58,59,63,65,67,70 (all completely); 64,69 (both partially)
	No:	Claims	NONE
Industrial applicability (IA)	Yes:	Claims	1-26,58,59,63,65,67,70 (all completely); 64 (partially)
	No:	Claims	NONE

2. Citations and explanations

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see s parate sh et

VIII. Certain observations on the int rnational application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Cited Documents

1. Reference is made to the following documents cited in the International Search Report:

   D1: WO 99 24578 A (PIZZA MARIAGRAZIA ;SCARLATO VINCENZO (IT); RAPPUOLI RINO (IT); CHI) 20 May 1999 (1999-05-20)

   D2: WIERTZ E J ET AL: 'T-cell responses to outer membrane proteins of Neisseria meningitidis: comparative study of the Opa, Opc, and PorA proteins.' INFECTION AND IMMUNITY, (1996 JAN) 64 (1) 298-304.

   D3: KIZIL G ET AL: 'Identification and characterization of TspA, a major CD4(+) T-cell- and B-cell-stimulating Neisseria -specific antigen.' INFECTION AND IMMUNITY, (1999 JUL) 67 (7) 3533-41.

   D4: NAESS L M ET AL: 'Human T-cell responses after vaccination with the Norwegian group B meningococcal outer membrane vesicle vaccine.' INFECTION AND IMMUNITY, (1998 MAR) 66 (3) 959-65.

2. The following documents (D) were not cited in the International Search Report.

   D5: SANDERSON S ET AL. J. Exp. Med. 1995. Vol. 182, Pp 1751-1757.

   D6: EP 0 287 206 A (National Research Development Corporation) 19.10.88.

Re Item I

Basis of the opinion

Description, claims, drawing sheet, and sequence listing as originally filed. Sequence listing pages 1 to 19 filed with the letter of 06.09.1999 do not form part of the application according to Rule 13ter.1(f) PCT.

Re Item II

Priority

1. The right of priority claimed **is not valid** for the subject-matter referred to in claims 60, 61, 62, 66, 68, and 71 completely; and 64 and 69 partially (**invention 5**, see below). The sequences SEQ ID NO 3 and SEQ ID NO 4 of the present application differ from the ones disclosed in the priority application GB 9814902.4 (filed on 10.07.1998). Therefore, **D1** and **D3** cited in the International Search Report as X,P documents are considered as prior art for the assessment of novelty and inventive step of the above cited claims which relate to SEQ ID NO 3 and SEQ ID NO 4 (invention 5).

2. In the present application the first recognized invention relates to a method of generating T-cell lines and cloned specific to the neisserial proteins, the method comprising isolating peripheral blood mononuclear cells. In the priority application, the same method was referring to peripheral blood lymphocytes. The term **"peripheral blood mononuclear cells" is broader than "peripheral blood lymphocytes"**, since it includes cells as monocytes. Thus, not all the cells referred to with the term "peripheral blood mononuclear cells" have the right of priority claimed.

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. **Claim 69** relates to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, **no opinion** will be formulated **with respect to the industrial applicability** of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

2. **Claims 72 to 79** are not clear, contrary to the criterion set forth in Article 6 PCT because the subject-matter for which protection is sought is not clearly defined.

These claims attempt to define the matter for which protection is sought merely by making reference "to the accompany drawings and sequences". Thus, the IPEA considers that it is not possible to carry out a meaningful examination of the subject-matter of claims 72 to 79.

Re Item IV

Lack of unity of invention

1. The International Preliminary Examination Authority considers that the present application lacks unity within the meaning of (Rule 13.1 PCT). The different recognized inventions (see list below) are no so linked as to form a single general inventive concept as a technical relationship involving one or more of the same or corresponding special technical features in the sense of Rule 13.2 PCT does not exist between the subject-matter of the different recognized inventions.

2. The common concept linking together the different recognized inventions is the use of neisserial proteins (or fragments thereof) in order to induce an immune response in human T-cells. **This concept is not new as D2** discloses human T-cell responses of different neisserial proteins (see abstract).

3. The separate inventions or groups of invention are:

   (i) **Invention 1 (claims 1 to 26 completely)** relates to a method of generating T-cell lines and clones specific to neisserial proteins, the method comprising isolating peripheral blood mononuclear cells (PBMCs) from the peripheral blood of normal donors and patients recovering from neisserial disease, culturing the PBMCs with neisserial proteins with or without a proliferation stimulant for a prescribed period, stimulating proliferation of T-cell lines and clones which are specific to neisserial proteins, and maintaining same by regular stimulation. It further relates (claims 19 to 26) to a method of detecting CD4+ T-cell stimulating proteins, the method comprising fractionating neisserial proteins and testing the ability of said proteins to stimulate proliferation of T-cell lines and

clones generated according to the method as claimed in any of claims 1 to 18.

(ii) **Invention 2 (claims 27 to 39 completely)** relates to a method of detecting CD4+ T-cell stimulating recombinant proteins, the method comprising screening a genomic meningococcal or gonococcal expression library for recombinant proteins which stimulate T-cell lines and clones.

(iii) **Invention 3 (claims 40 to 57 completely)** relates to a method of detecting CD4+ T-cell stimulating peptides, the method comprising screening meningococcal or gonococcal genomic phage display libraries (PDLs) to identify peptides which stimulate T-cell lines and clones.

(iv) **Invention 4** (see below).

(v) **Invention 5** (see below).

4. The right of priority claimed **is not valid** (see item II of the present report) for the subject-matter referred to in claims 60, 61, 62, 66, 68, and 71 (all completely); and 64, 69, and 72 to 79 (all partially). The sequences SEQ ID NO 3 and SEQ ID NO 4 of the present application differ from the ones disclosed in the priority application GB 9814902.4 (filed on 10.07.1998). Therefore, **D1** and **D3** cited in the International Search Report as X,P documents are considered as prior art for the assessment of novelty and inventive step of the above cited claims which relate to SEQ ID NO 3 and SEQ ID NO 4.

D1 discloses DNA and amino acid sequences from *Neisseria meningitidis* and *N. gonorrhoeae* useful for diagnosis, treatment and prevention of the infection. Sequence ID NO 267 has 96.2 % identity in 260 bp overlap with SEQ ID NO 3 of the present application.

D3 discloses the identification and characterization of TspA, a major CD4+ T-Cell and B-Cell-stimulating Neisseria-specific antigen. In search for novel T-cell immunogens involved in protection against invasive meningococcal disease, fractionated proteins of *Neisseria meningitidis* were screened by using peripheral blood mononuclear cells

and specific T-cell lines obtained from normal individuals and patients convalescing from *N. meningitidis* infection.

Therefore, as the part of the application relating to SEQ ID NO 3 and SEQ ID NO 4 have not the right of priority, and D1 and D3 would have provide a skilled person with the tools to identify Neisserial sequences which would give protection against meningococcal disease, the IPEA considers that there is further non-unity in respect with the parts of the application relating to the SEQ ID NOs 1 and 2, and SEQ ID NOs 3 and 4. Therefore, other two additional inventions or groups of inventions are recognized:

(iv) **Invention 4 (claims 58, 59, 63, 65, 67, and 70 completely; and 64 and 69 partially)** relates to a nucleotide sequence comprising a base sequence as shown in SEQ ID NO 1, or an active derivative thereof, the sequence coding for a polypeptide having an amino acid sequence as shown in SEQ ID NO 1 and SEQ ID NO 2, or an active derivative thereof; purified and isolated DNA composites comprising SEQ ID NO 1, or an active derivative thereof; the polypeptide or an active fragment thereof encoded by such sequences; the use of such polypeptides in the manufacture of vaccines against neisserial disease; compositions for use as vaccines against neisserial disease: a vaccine against neisserial disease comprising polypeptide with some or all of the amino acid sequence as shown in SEQ ID NO 2; and a method of treatment of neisserial disease comprising inducing T-cell proliferation with polypeptide comprising the amino acid sequence shown in SEQ ID NO 2 or an active derivatives thereof.

(v) **Invention 5 (claims 60, 61, 62, 66, 68, and 70 completely; and 64 and 69 partially)** relates to a nucleotide sequence comprising a base sequence as shown in SEQ ID NO 3, or an active derivative thereof, the sequence coding for a polypeptide having an amino acid sequence as shown in SEQ ID NO 3 and SEQ ID NO 4, or an active derivative thereof; purified and isolated DNA composites comprising SEQ ID NO 3, or an active derivative thereof; the polypeptide or an active fragment thereof encoded by such sequences; the use of such polypeptides in the manufacture of vaccines against neisserial disease; compositions for use as vaccines against neisserial disease: a vaccine against neisserial disease comprising polypeptide with some or all of the amino acid

sequence as shown in SEQ ID NO 4; and a method of treatment of neisserial disease comprising inducing T-cell proliferation with polypeptide comprising the amino acid sequence shown in SEQ ID NO 4 or an active derivatives thereof.

5. The Applicant was invited either to restrict the claims to only one of the recognized inventions or to pay an additional fee for each additional invention claimed. The Applicant elected to pay additional fees without protest for the examination of additional inventions.

Thus, the IPEA has carried out examination on the subject-matter of inventions for which examination fees were paid, corresponding to the subject-matter of inventions 1 and 4 (claims 1 to 26, 58, 59, 63, 65, 67, 70 [all completely], and 64 and 69 [both partially]).

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

INVENTION 1

1 Invention 1 meets the PCT requirements as subject-matter of **claims 1 to 26 is novel, involves an inventive step**, and is **susceptible of industrial application, in view of the prior art available to the IPEA.**

D4 discloses the analysis of human T-cell responses after vaccination with a meningococcal outer membrane vesicle (OMV) vaccine. Adult volunteers (with no or very low levels of serum IgG antibodies against meningococci) received three doses intramuscularly of the OMV vaccine (at weeks 0, 6, and 46). PBMCs were isolated from whole blood by density centrifugation and assayed for in vitro proliferative responses by the thymidine incorporation method (after 6 days of incubation, see page 960, left column, second paragraph) against the OMV vaccine, purified OMPs (PorA and PorB), and control antigens (*Mycobacterium bovis* BCG vaccine, and tetanus toxoid) before and after vaccination (see abstract, and page 960, left column,

first paragraph).

The T-cell responses were compared with the corresponding OMV-specific antibody concentrations in serum, and changes in the PBMC subsets during the vaccination schedule against meningococci were studied (see page 962, right column, lines 8 to 11). The OMV vaccine induced antigen-specific T-cell responses, and vaccination increased the proportion of memory CD4+ T-cells (see abstract).

In D4, the sources of PBMCs were adult volunteers (with no or very low levels of serum IgG antibodies against meningococci) before vaccination with the OMV vaccine, and after vaccination with such vaccine. Therefore, in D4 patients recovering from neisserial disease were not used as a source of PBMCs.

In the method of generating T-cell lines specific to neisserial proteins of invention 1 of the present application, the PBMCs were isolated from the peripheral blood from normal donors and patients recovering from neisserial disease, these PBMCs were cultured with neisserial proteins, stimulating the proliferation of T-cell lines and clones which are specific to neisserial proteins, and the T-cell lines were maintained by regular stimulation.

Therefore, it appears that invention 1 deals with the generation of a T-cell library that can be used in identifying natural neisserial peptide antigens which are T-cell stimulants. These T-cell clones which were obtained from patients recovering from neisserial disease and which proliferate in the presence of neisserial proteins, would allow to detect neisserial antigens.

D4 discloses that vaccination with OMV vaccine induces T-cell proliferation. Therefore, it is known that in patients recovering from neisserial disease the proliferation of specific T-cell lines is induced in response to the specific antigens of the neisserial disease-inducing microorganism.

However, neither D2 nor D4 deal with the establishment of T-cell libraries from patients recovering from neisserial disease (involving g neration of the T-cell lin s and maintenance of said T-cell lines by regular stimulation) in order to provide a system to detect natural neisserial antigens. There is no indication

in these two documents to use such a method to detect antigens, and therefore, the subject-matter of claim 1 appears to be novel, to involve and inventive step, and to be susceptible of industrial application.

2. Although the use of iron restricted growth in order to enhance immunogenecity of bacterial proteins was known in the prior art (see **D6**), claim 2 also meets the requirements of the PCT since it refers to the method of claim 1. Also claims 3 to 18 meet the requirements of the PCT since they refer to claim 1.

3. Also claims 19 to 26 meet the requirements of the PCT.

3.1 Both purified neisserial proteins, and fractions containing neisserial proteins (OMV vaccine) were disclosed to increase the percentage of CD4+ cells, (see D2 and D4).

D2 discloses a comparative study of the T-cell responses of the Opa, Opc, and PorA proteins from *Neisseria meningitidis*. It discloses that different neisserial proteins have different abilities to stimulate proliferation of T cells. In order to investigate the relative immunodominance of class 5 outer membrane proteins (Opa and Opc proteins), purified Opa, Opc, and synthetic peptides derived from class 5 protein sequences were tested for recognition by T cells obtained from the peripheral blood of human volunteers (see page 298, right column, second paragraph). It would have been obvious to fractionate complex protein fractions, in order to identify the most active components.

3.2 **D5** discloses a method for generating and detecting CD4+ T-cell stimulating proteins, the method comprising screening a genomic expression library for recombinant proteins which stimulate T-cell lines and clones. T-cell lines and clones specific to LM proteins were generated by isolating CD4+ T-cells from immunized mice. D5 discloses the identification of a CD4+ T-cell stimulating antigen of pathogenic bacteria (*Listeria monocytogenes, **LM***) by expression cloning. Using ***LM***-specific, lacZ-inducible T cells as single-cell probes, a **LM** genomic library was screened as recombinant *Escherichia coli* that was fed to macrophages. The antigen gene was isolated from the *E.coli* gene that, when ingested by the macrophages, allowed

generation of the appropriate peptide/MHC class II complex and T cell activation (see abstract).

3.3 However, claims 19 to 26 meet the criteria of the PCT since they refer to the method of claim 1 to generate T-cell lines and clones specific to neisserial proteins.

INVENTION 4

1 Claims 58, 59, 63, 65, 67, and 70 (all completely) and 64 (partially) relate to compositions, uses and a method involving the sequences SEQ ID NO: 1 and SEQ ID NO:2. **The IPEA considers that these claims are novel, inventive and susceptible of industrial applicability.**

2. **Claim 69 is also novel and inventive**, but this claim relates to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

For the assessment of the present claim 69 on the question whether ii is industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item VII

Certain defects in the international application

1. The present application does not meet the requirements of the PCT (see International Preliminary Examination Guidelines, Section IV, III-4.3a), because on page 19 of the description (second paragraph) there are general statements which

imply that the extent of the protection may be expanded in a not precisely defined way.

2. When referring to documents cited in the description, the applicant indicates the first author, and the year (and occasionally an internal reference number), but without indicating the journal, volume and pages, and no list of references with proper citations is present on file.

Re Item VIII

Certain observations on the international application

1. **Claims 72 to 79** are not clear (see also Re Item III) contrary to the criterion set forth in Article 6 PCT because the subject-matter for which protection is sought is not clearly defined. Furthermore, according to Rule 6.2 (a) PCT, claims shall not, except when absolutely necessary rely, in respect of the technical features of the invention, on references to the description or drawings. In particular, they shall not rely on such references as: "... as hereinbefore described with reference to the accompany drawing and sequences".

INVENTION 4

2. **Claims 58, 59, 63, 65, 67, and 70 (all completely), and 64 and 69 (both partially) are not clear**, contrary to the criterion set forth in Article 6 PCT, because the subject-matter for which protection is sought is not clearly defined. These claims refer to DNA constructs, nucleotide sequences, DNA compositions, polypeptides, the use of such polypeptides, and a method of treatment, comprising sequences as shown in SEQ ID NOs:1 and 2 or **"active derivatives thereof"**. The expression **"active derivatives thereof"** is not clearly defined, thus rendering the scope of all these claims unclear.

The IPEA has considered that the expression "active derivatives thereof" refers to derivatives which have the same **immunogenic activity** as the one shown by the DNA and protein represented by SEQ ID NOs: 1 and 2.

3. **Claim 59 is further unclear.** This claim refers to "a polypeptide as claimed in claim 58, characterized in that the polypeptide is a CD4+ T-cell stimulant". Claim 58 relates to "the use of a polypeptide in the manufacture of a vaccine against neisserial disease". As claim 58 is directed to "the use of a polypeptide", and claim 59 refers to "a polypeptide as claimed in claim 58", it is not clear wether claim 59 is a "use claim" or a "product claim".

CLAIMS

1. A method of generating T-cell lines and clones specific to neisserial proteins, the method comprising isolating peripheral blood mononuclear cells (PBMCs) from the peripheral blood of normal donors and patients recovering from neisserial disease, culturing the PBMCs with neisserial proteins with or without a proliferation stimulant for a prescribed period, stimulating proliferation of T-cell lines and clones which are specific to neisserial proteins, and maintaining same by regular stimulation.

2. A method as claimed in claim 1, characterised in that the neisserial proteins are prepared from *Neisseria meningitidis* and/or *Neisseria gonorrhoea* grown under iron restrictions to induce the expression of iron-regulated proteins.

3. A method as claimed in any preceding claim, characterised in that the peripheral blood is obtained from naturally infected patients at different stages of illness.

4. A method as claimed in claim 3, characterised in that the stages include an acute stage (on admission), early convalescence (seven days after admission), late convalescence (six weeks after discharge) and after full recovery (3 months and twelve months after discharge).

5. A method as claimed in any preceding claim, characterised in that the peripheral blood is heparinised or treated with ESTA.

6. A method as claimed in any preceding claim, characterised in that the PBMCs are isolated from the blood by centrifugation.

7. A method as claimed in any preceding claim, characterised in that the PBMCs are initially cultured in medium containing human serum.

8. A method as claimed in any preceding claim, characterised in that the PBMCs are cultured with the neisserial proteins and Interleukin 2 (IL-2) for a predetermined period.

9. A method as claimed in claim 8, characterised in that the predetermined period is 3-10 days and may be 5 days.

10. A method as claimed in any of claims 8 or 9, characterised in that IL-2 stimulates the proliferation of the activated T-cell lines and clones.

11. A method as claimed in claim 10, characterised in that the T-cell lines and clones are maintained by weekly stimulation.

12. A method as claimed in claim 10 or claim 11, characterised in that the stimulation is provided by proteins in the presence of IL-2 and feeder cells.

13. A method as claimed in claim 12, characterised in that the feeder cells are antigen presenting feeder cells and may be autologous Epstein-Barr virus transformed B-lymphocytes (EBVB).

14. A method as claimed in any preceding claim, characterised in that the specificity of the T-cell lines and clones to neisserial proteins is tested prior to storing for example in liquid nitrogen.

15. A method as claimed in claim 14, characterised in that the specificity is tested by measurement of tritiated thymidine incorporation in response to stimulation with neisserial proteins compared to irrelevant antigens.

16. A method as claimed in claim 15, characterised in that an irrelevant antigen is tetanus toxoid.

17. A method as claimed in any preceding claim, characterised in that the phenotype of the T-cell lines and clones are also assessed using flow cytometry

and specific monoclonal antibodies.

18. A method as claimed in claim 17, characterised in that the antibodies are $CD4^+$, $CD8^-$ and α/β- and γ/δ- T-cell receptor (TCR) specific monoclonal antibodies.

19. A method of detecting $CD4^+$ T-cell stimulating proteins, the method comprising fractionating neisserial proteins and testing the ability of said proteins to stimulate proliferation of Neisseria specific T-cell lines and clones generated according to the method as claimed in any of the preceding claims.

20. A method as claimed in claim 19, characterised in that the proteins are fractionated by SDS-PAGE.

21. A method as claimed in any of claims 19 or 20, characterised in that the fractions are tested for their ability to stimulate the individual T-cell lines and clones.

22. A method as claimed in claim 21, characterised in that fractions containing T-cell stimulants are further characterised by SDS-PAGE.

23. A method as claimed in any of claims 19 to 22, characterised in that polyclonal antibodies are raised to the T-cell stimulating fraction proteins.

24. A method as claimed in claim 23, characterised in that the antibodies are used to screen a genomic meningococcal and/or gonococcal expression library.

25. A method as claimed in claim 24, characterised in that the expression library is a λZapII library.

26. A method as claimed in claim 24 or claim 25, characterised in that isolated neisserial polypeptides which react with the antibodies and their respective DNA fragments are further characterised and sequenced.

27. A method of detecting CD4+ T-cell stimulating recombinant proteins, the method comprising screening a genomic meningococcal or gonococcal expression library for recombinant proteins which stimulate T cell lines and clones.

28. A method as claimed in claim 27, characterised in that the T-cell lines and clones are meningococcal and/or gonococcal specific T-cell lines and clones generated according to the method of any of claims 1 to 18.

29. A method as claimed in claim 27 or claim 28, characterised in that the genomic meningococcal or gonococcal expression library is a λZapII phage library expressing genomic DNA extracted from a strain of *Neisseria meningitidis* or a strain of *Neisseria gonorrhoea*.

30. A method as claimed in claim 29, characterised in that a representative pool of recombinant pBluescript SKII plasmid are excised from the phage library and transformed into *E.coli* strain XL1-Blue.

31. A method as claimed in claim 30, characterised in that the plasmids are excised into XL1-Blue using a helper phage.

32. A method as claimed in claim 30 or claim 31, characterised in that the transformed *E.coli* are cultured in a medium which may contain ampicillin.

33. A method as claimed in any of claims 27 to 32, characterised in that meningococcal or gonococcal protein expression is induced by isopropyl-b-D-thio-galactoside.

34. A method as claimed in any of claims 27 to 33, characterised in that the bacteria are heat-killed and sonicated before adding to antigen presenting cells.

35. A method as claimed in any of claims 27 to 34, characterised in that the expressed proteins are tested for their ability to stimulate the individual T-cell

lines and clones.

36. A method as claimed in any of claims 27 to 35, characterised in that CD4+ T-cell stimulating bacterial cultures are identified and subcultured.

37. A method as claimed in claim 36, characterised in that the subcultures are preferably rescreened for T-cell stimulation.

38. A method as claimed in claim 36 or claim 37, characterised in that the CD4+ T-cell stimulants are identified by sequencing and are further characterised.

39. A method as claimed in any of claims 27 or 28, characterised in that the genomic meningococcal or gonococcal expression library is a λZapII phage library expressing genomic DNA extracted from a meningococcal or gonococcal genomic lambda phage display library.

40. A method of detecting CD4+ T-cell stimulating peptides, the method comprising screening meningococcal or gonococcal genomic phage display libraries (PDLs) to identify peptides which stimulate T-cell lines and clones.

41. A method as claimed in claim 40, characterised in that the T-cell lines and clones are meningococcal and/or gonococcal specific T-cell lines and clones generated according to the method as claimed in any of claims 1 to 18.

42. A method as claimed in any of claims 39 to 41, characterised in that the genomic phage display library (PDL) is generated by fragmenting bacterial DNA, cloning and packaging into bacteriophage vectors.

43. A method as claimed in claim 42, characterised in that two vectors are used.

44. A method as claimed in claim 43, characterised in that the first vector

displays peptides up to 1200 amino acids which are expressed at low copy numbers.

45. A method as claimed in claim 43 or claim 44, characterised in that the second vector preferably displays up to 415 copies of a peptide up to 50 amino acids in size.

46. A method as claimed in any of claims 40 to 45, characterised in that the PDLs are amplified in respective *E.coli* hosts.

47. A method as claimed in any of claims 40 to 46, characterised in that the cells are heat killed before testing for the ability of the peptides to stimulate the T-cell lines and clones.

48. A method as claimed in any of claims 40 to 47, characterised in that $CD4^+$ T-cell stimulating PDL cultures are identified and subcultured.

49. A method as claimed in claim 48, characterised in that the subcultures are rescreened for T-cell stimulation.

50. A method as claimed in any of claims 40 to 49, characterised in that the $CD4^+$ T-cell stimulants are identified by sequencing and are further characterised.

51. A method of detecting $CD4^+$ T-cell stimulating recombinant proteins, using a meningococcal or gonococcal genomic lambda phage display library in accordance with any of claims 27 to 39.

52. A method as claimed in claim 51, characterised in that the meningococcal or gonococcal genomic lambda phage display library is constructed by cloning randomly amplified PCR products using two random primers, each tagged at 5' end to restriction sites, inserting same into a pre-digested vector, and plating by infecting *E.coli*.

53. A method as claimed in claim 52, characterised in that the vector is a lambda phage.

54. A method as claimed in claim 53, characterised in that the vector is λpRH825 vector.

55. A method as claimed in claim 53 or 54, characterised in that the amplified and digested DNA fragments are packaged into the lambda phage using a lambda phage packaging kit.

56. A method as claimed in any of claims 52 to 55, characterised in that the restriction sites are SpeI or NotI.

57. A method as claimed in any of claims 51 to 56, characterised in that the DNA inserts in the plaques formed are sequenced, thereby confirming that the plaques contain DNA fragments of meningococcal or gonococcal origin.

58. Use of a polypeptide in the manufacture of a vaccine against neisserial disease, the peptide comprising an amino acid sequence as shown in SEQIDNO1 and SEQIDNO2 or an active derivative thereof.

59. A polypeptide as claimed in claim 58, characterised in that the polypeptide is a $CD4^+$ T-cell stimulant.

60. A DNA construct for use in the manufacture of a medicament for the treatment of neisserial disease the construct comprising a sequence as shown in SEQIDNO3 or an active derivative thereof.

61. Use of a polypeptide in the manufacture of a vaccine against neisserial disease, the peptide comprising an amino acid sequence as shown in SEQIDNO3 and SEQIDNO4 or an active derivative thereof.

62. A polypeptide as claimed in claim 61, characterised in that the

polypeptide is a CD4+ T-cell stimulant.

63. A DNA construct for use in the manufacture of a medicament for the treatment of neisserial disease, the construct comprising a sequence as shown in SEQIDNO1, or an active derivative thereof.

64. A composition for use as a vaccine against neisserial disease, the composition comprising two peptides with the amino acid sequences as shown in SEQIDNO1 and SEQIDNO2, and SEQIDNO3 and SEQIDNO4 or active derivatives thereof.

65. A nucleotide sequence comprising a base sequence as shown in SEQIDNO1, or an active derivative thereof, the sequence coding for a polypeptide having an amino acid sequence as shown in SEQIDNO1 and SEQIDNO2, or an active derivative thereof.

66. A nucleotide sequence comprising a base sequence as shown in SEQIDNO3, or an active derivative thereof, the sequence coding for a polypeptide having an amino acid sequence as shown in SEQIDNO3 and SEQIDNO4, or an active derivative thereof.

67. A vaccine against neisserial disease, the vaccine comprising polypeptide with the amino acid sequence as shown in SEQIDNO2 or an active derivative thereof.

68. A vaccine against neisserial disease, the vaccine comprising polypeptide with the amino acid sequence as shown in SEQIDNO4 or an active derivative thereof.

69. A method of treatment of neisserial disease, the method comprising inducing T-cell proliferation with polypeptide comprising one or both of the amino acid sequences shown in SEQIDNO2 and SEQIDNO4, or active derivative(s) thereof.

70. A purified and isolated DNA composite comprising the sequence shown in SEQIDNO1, or an active derivative thereof.

71. A purified and isolated DNA composition comprising the sequence shown in SEQIDNO3, or an active derivative thereof.

72. A methodology substantially as hereinbefore described with reference to the accompany drawings and sequences.

73. Use of a polypeptide substantially as hereinbefore described with reference to the accompany drawings and sequences.

74. A DNA construct substantially as hereinbefore described with reference to the accompany drawings and sequences.

75. A composition substantially as hereinbefore described with reference to the accompany drawings and sequences.

76. A nucleotide sequence substantially as hereinbefore described with reference to the accompany drawings and sequences.

77. A vaccine substantially as hereinbefore described with reference to the accompany drawings and sequences.

78. A method of treatment substantially as hereinbefore described with reference to the accompany drawings and sequences.

79. Any novel subject matter or combination including novel subject matter disclosed herein, whether or not within the scope of or relating to the same invention as any of the preceding claims.

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(54) Title: SCREENING OF NEISSERIAL VACCINE CANDIDATES AND VACCINES AGAINST PATHOGENIC NEISSERIA

(57) Abstract

Methods of screening for vaccine candidates, vaccines against pathogenic neisseria and intermediaries for such vaccines have been developed. Two vaccine candidates TspA and TspB have been identified and characterised which either alone or in conjunction with the vaccines provide for treatment against pathogenic neisserias in particular Neisseria meningitidis and/or Neisseria gonorrhoea.

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INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
NW/6943INT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 99/ 02205	09/07/1999	10/07/1998

Applicant

THE UNIVERSITY OF NOTTINGHAM et al.

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[X] contained in the international application in written form.

[X] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readble form.

[X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of Invention is lacking** (see Box II).

4. With regard to the **title,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

[ ] as suggested by the applicant.

[ ] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

[X] None of the figures.

INTERNATIONAL SEARCH REPORT

ernational application No.
PCT/GB 99/02205

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 70 and 79 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No
[redacted]/GB 99/02205

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N5/08 G01N33/50 C12Q1/68 A61K39/095 C07K14/22
C12N15/31

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	KIZIL G ET AL: "Identification and characterization of TspA, a major CD4(+) T-cell- and B-cell-stimulating Neisseria -specific antigen." INFECTION AND IMMUNITY, (1999 JUL) 67 (7) 3533-41. , XP002127425 the whole document ---	1-65,68, 69, 73-76, 78-80
X,P	WO 99 24578 A (PIZZA MARIAGRAZIA ;SCARLATO VINCENZO (IT); RAPPUOLI RINO (IT); CHI) 20 May 1999 (1999-05-20) page 3, line 15 - line 28; claims 4-6,8-17; example 32 page 4, line 7 - line 18 page 5, line 3 - line 16 ---	61,63, 65,67, 72-78,80
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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Date of the actual completion of the international search	Date of mailing of the international search report
14 January 2000	28/01/2000

Name and mailing address of the ISA	Authorized officer
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2

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	NAESS L M ET AL: "Human T-cell responses after vaccination with the Norwegian group B meningococcal outer membrane vesicle vaccine." INFECTION AND IMMUNITY, (1998 MAR) 66 (3) 959-65. , XP002127426 page 959, column 1, paragraph 1 -column 2, paragraph 1 page 962, column 2, paragraph 1 page 964, column 1, paragraph 4 ---	1-27
A	WIERTZ E J ET AL: "T-cell responses to outer membrane proteins of Neisseria meningitidis: comparative study of the Opa, Opc, and PorA proteins." INFECTION AND IMMUNITY, (1996 JAN) 64 (1) 298-304. , XP002127427 page 298, column 2, paragraph 2 page 300, column 1, paragraph 1 page 303, column 1, paragraph 1 -----	1-27

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 99/02205

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9924578 A	20-05-1999	AU 9363798 A	31-05-1999